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(54) Title: AMYLIN ANTAGONISTS AND AGONISTS (57) Abstract The invention features amylin analogs which behave as amylin antagonists and agonists. The invention also features the use of the amylin antagonist for the treatment of Type II diabetes mellitus, and the use of the amylin agonists for the treatment of both Type I diabetes mellitus and hypercalcemia. The invention also features the use of amylin antagonists and agonists for the control of food intake.		

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AMYLIN ANTAGONISTS AND AGONISTSBackground of the Invention

This invention relates to specific amylin analogs
5 which behave as amylin antagonists and agonists, and to
their use in the treatment of diabetes mellitus, and
hypercalcemia, and the control of food intake.

Amylin, also known as diabetes associated
polypeptide (Cooper et al., Proc. Natl. Acad. Sci. USA,
10 85:7763-7766 (1988)) or islet/insulinoma amyloid
polypeptide (Westermarck et al., Proc. Natl. Acad. Sci.
USA, 84:3881-3885 (1987)), is a 37-residue polypeptide
amide isolated originally from the amyloid-rich pancreas
of insulinoma and noninsulin-dependent diabetic (NIDD)
15 patients. It has subsequently been isolated from the
normal pancreas of rat (Asai et al., Biochem. Biophys.
Res. Commun., 164:400-405 (1989)). CDNA cloning (Ferrier
et al., J. Mol. Endocrinol., 3:R1-R4 (1989)) and
immunocytochemical (Lukinius et al., Diabetologia,
20 32:240-244 (1989)) studies have demonstrated that amylin
is synthesized in the islet cells and stored in the islet
secretory granules along with insulin. It is cosecreted
with insulin (Kanatsuka et al., FEBS Lett., 259:199-201
(1989)). Low quantities of amylin have also been
25 detected in the stomach, intestine, lung and dorsal root
ganglion (Asai et al., Biochem. Biophys. Res. Commun.,
169:788-795 (1990)); and Ferrier et al., supra).

Biological investigations that followed the
isolation of amylin have shown that amylin inhibits basal
30 and insulin-stimulated glucose uptake as well as glycogen
synthesis by soleus muscles (Leighton et al., Nature,
335:632-635 (1988)). This peripheral insulin resistance
by amylin has also been demonstrated in vivo by
euglycemic glucose clamp studies with dogs (Sowa et al.,

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Diabetologia, 33:118-120 (1990)) and rats (Molina et al., Diabetes, 39:260-265 (1990)). Furthermore, these investigations in rats showed that amylin attenuated the inhibition of hepatic glucose output by insulin (Molina et al., supra). Based on these observations and the finding that amylin inhibits basal insulin secretion (Ohsawa et al., Biochem. Biophys. Res. Commun., 160:961-967 (1989)), it has been suggested that amylin might play a role in glucose metabolism and the pathophysiology of noninsulin-dependent diabetes mellitus (NIDDM), commonly known as Type II diabetes mellitus.

Diabetes mellitus is a metabolic disease characterized by chronic hyperglycemia, i.e., elevated blood sugar levels. This disease affects a significant percentage of the population. There are two major categories of diabetes mellitus, commonly referred to as Type I and Type II. In patients with Type I diabetes mellitus, there is a loss of active β -cells in the islets of Langerhans in the pancreas, resulting in low levels of both insulin and amylin. Cooper, Medical Hypothesis, 26:284-288 (1991). Patients with Type I diabetes mellitus who are treated with insulin frequently have a tendency to develop hypoglycemia as a side effect. In patients with Type II diabetes mellitus, there are elevated levels of amylin. Patients with type II diabetes mellitus display varying resistance to the normal biological effects of insulin. Increased levels of amylin, known as hyperamylinemia, have been implicated in causing insulin resistance in a number of model systems, including genetically obese LA/N-cp rats (Huang et al., Hypertension, 19:i-101 - i-109 (1992)), genetically obese diabetic yellow mice (Gill et al., Life Sci, 48:703-718 (1991)), dexamethasone induced diabetic rats (Jamal et al., J. Endocrin., 126:425-429 (1990)), streptozocin induced diabetic rats (Inoue et al.,

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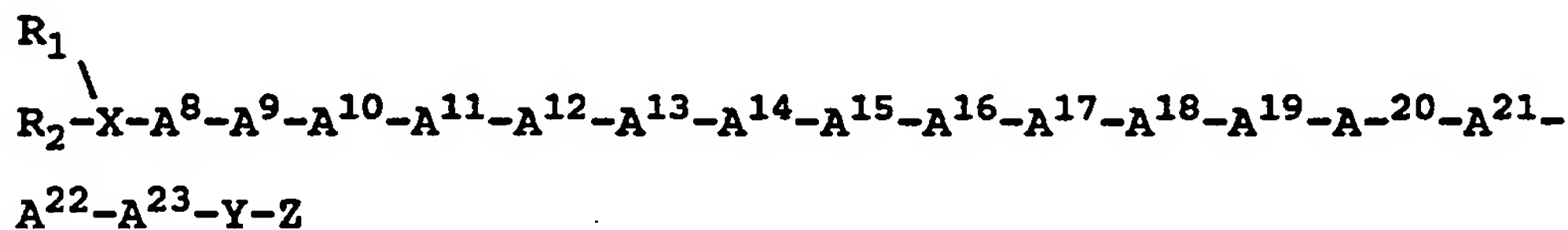
Diabetes, 41:723-727 (1992)), and ventromedial hypothalamic lesioned rats and Zucker rats (Tokuyama et al., Endocrinology, 128:2739-2744 (1991)).

Other studies have shown that amylin, like
 5 calcitonin, can exhibit serum calcium-lowering effects in rats in vivo as well as in cell culture systems (Datta et al., Biochem. Biophys. Res. Commun., 162:876-881 (1989)). Amylin has also been shown to act as an anorectic agent. Balasubramaniam et al., Peptides, 12:919-924 (1991).

10 Summary of the Invention

In general, the invention features amylin analogs which behave as amylin antagonists and agonists.

In one aspect, the invention features amylin analogs which are linear analogs of biologically active
 15 amylin having the following amino acid formula:



20 wherein:

X is a chain of 0-5 amino acids, inclusive, the N-terminal one of which is bonded to R₁ and R₂;

25 Y is a chain of 0-4 amino acids, inclusive, the C-terminal one of which is bonded to Z;

Each of R₁, and R₂, independently, is H, C₁-C₁₂ alkyl (e.g., methyl), C₆-C₁₈ aryl (e.g., phenyl, naphthaleneacetyl), C₁-C₁₂ acyl (e.g., formyl, acetyl, and myristoyl), C₇-C₁₈ aralkyl (e.g., benzyl), or C₇-C₁₈ alkaryl (e.g., p-methylphenyl);
 30

A⁸ is Ala, Nal, Thi, Phe, Bth, Pcp, N-Me-Thr, Aib, or Anb;

35 A⁹ is Thr, Ala, Anb, Aib, Ser, N-Me-Ser, or N-Me-Thr;

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A¹⁰ is Gln, Ala, Asn, N-Me-Gln, Gly, Nva, Aib, or Anb;

5 A¹¹ is Arg, homo-Arg, diethyl-homo-Arg, Lys- ϵ -NH-R (where R is H, a branched or straight chain C₁-C₁₀ alkyl group, or an aryl group), Orn, or Lys;

A¹² is Leu, Ile, Val, Aib, Anb, or N-Me-Leu;

A¹³ is Ala, Nal, Thi, Phe, Bth, Pcp, N-Me-Thr, Aib, or Anb;

10 A¹⁴ is Asn, Ala, Gln, Gly, N-Me-Asn, Nva, Aib, or Anb;

A¹⁵ is Phe, or any aromatic amino acid with or without substituents;

A¹⁶ is Leu, Ile, Val, Aib, Anb, or N-Me-Leu;

15 A¹⁷ is Val, Ile, Aib, Anb, or N-Me-Val;

20 A¹⁸ is His, Thr, 3-Me-His, 1-Me-His, β -pyroazolylalanine, N-Me-His, Arg, homo-Arg, diethyl-homo-Arg, Lys- ϵ -NH-R (where R is H, a branched or straight chain C₁-C₁₀ alkyl group, or an aryl group), Ala, Aib, Anb, or Orn;

A¹⁹ is Ser, Thr, N-Me-Ser, N-Me-Thr, Aib, Anb, or Ala;

25 A²⁰ is Ser, Thr, N-Me-Ser, N-Me-Thr, Aib, Anb, or Ala;

A²¹ is Asn, Ala, Gln, Gly, N-Me-Asn, Aib, Anb, or Nva;

A²² is Asn, Ala, Gln, Gly, N-Me-Asn, Aib, Anb, or Nva;

30 A²³ is Phe, any aromatic amino acid with or without substituents, Leu, Ile, Val, Aib, Anb, Ala, or N-Me-Leu; and

35 Z is NHR₃ or OR₃; wherein R₃ is H, C₁-C₁₂ alkyl, C₇-C₁₀ phenylalkyl, C₃-C₂₀ alkenyl, C₃-C₂₀ alkynyl, phenyl, or naphthyl.

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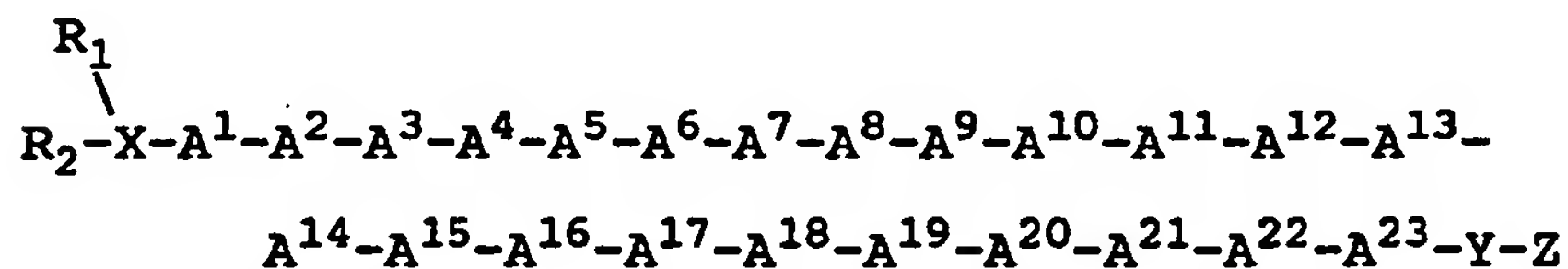
In preferred embodiments, the analogs are antagonists.
 In a highly preferred embodiment, the amylin antagonist
 corresponds to the N- α acetyl derivative of amino acids 8
 through 23 of human amylin with an amidated carboxy at
 5 the C-terminus, referred to herein as N- α -ac-human amylin
 (8-23)-NH₂, having the following formula:

N- α -Ac-Ala-Thr-Gln-Arg-Leu-Ala-Asn-Phe-Leu-Val-His-Ser-
 Ser-Asn-Asn-Phe-NH₂ (SEQ ID NO:1)

In another preferred embodiment, the amylin antagonist
 10 has the following formula:

N- α -Ac-Ala-Thr-Gln-Arg-Leu-Ala-Asn-Phe-Leu-Val-Arg-Ser-
 Ser-Asn-Asn-Leu-NH₂. (SEQ ID NO:2)

In another aspect, the invention features amylin
 analogs which are linear analogs of biologically active
 15 amylin having the following amino acid formula:



20 wherein

X is a chain of 0-5 amino acids, inclusive,
 the N-terminal one of which is bonded to R₁
 and R₂;

25 Y is a chain of 0-4 amino acids, inclusive,
 the C-terminal one of which is bonded to Z;

Each of R₁, and R₂, independently, is H,
 C₁-C₁₂ alkyl, C₆-C₁₈ aryl, C₁-C₁₂ alyl,
 C₇-C₁₈ aralkyl, or C₇-C₁₈ alkaryl;

30 A¹ is Lys, Arg, homo-Arg, diethyl-homo-Arg,
 Lys- ϵ -NH-R (where R is H, a branched or
 straight chain C₁-C₁₀ alkyl group, or an aryl
 group), or Orn;

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- 5 A² is Cys, or Anb;
- A³ is Asn, Ala, Gln, Gly, N-Me-Asn, Aib, Anb,
 or Nva;
- A⁴ is Thr, Ser, N-Me-Ser, N-Me-Thr, Ala, Aib,
 or Anb;
- A⁵ is Ala, Nal, Thi, Phe, Bth, Pcp, N-Me-Ala,
 Aib, or Anb;
- A⁶ is Thr, Ser, N-Me-Ser, N-Me-Thr, Ala, Aib,
 or Anb;
- 10 A⁷ is Cys, or Anb;
- A⁸ is Ala, Nal, Thi, Phe, Bth, Pcp, N-Me-Ala,
 Aib, or Anb;
- A⁹ is Thr, Ser, N-Me-Ser, N-Me-Thr, Ala, Aib,
 or Anb;
- 15 A¹⁰ is Gln, Ala, Asn, N-Me-Gln, Gly, Nva,
 Aib, or Anb;
- A¹¹ is Arg, homo-Arg, diethyl-homo-Arg, Lys-
 ε-NH-R (where R is H, a branched or straight
 chain C₁-C₁₀ alkyl group, or an aryl group),
20 or Orn;
- A¹² is Leu, Ile, Val, Aib, Anb, or N-Me-Leu;
- A¹³ is Ala, Nal, Thi, Phe, Bth, Pcp, N-Me-
 Ala, Aib, or Anb;
- A¹⁴ is Asn, Ala, Gln, Gly, N-Me-Asn, Aib,
25 Anb, or Nva;
- A¹⁵ is Phe, or any aromatic amino acid with
 or without substituents;
- A¹⁶ is Leu, Ile, Val, Aib, Anb, or N-Me-Leu;
- A¹⁷ is Val, Ile, Aib, Anb, or N-Me-Val;
- 30 A¹⁸ is His, Thr, 3-Me-His, 1-Me-His, β-
 pyrolylalanine, N-Me-His, Arg, homo-Arg,
 diethyl-homo-Arg, Lys-ε-NH-R (where R is H, a
 branched or straight chain C₁-C₁₀ alkyl
 group, or an aryl group), Orn, Ala, Aib, or
35 Anb;

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A¹⁹ is Ser, Thr, N-Me-Ser, N-Me-Thr, Ala, Aib, or Anb;

A²⁰ is Ser, Thr, N-Me-Ser, N-Me-Thr, Ala, Aib, or Anb;

5 A²¹ is Asn, Ala, Gln, Gly, N-Me-Asn, Aib, Anb, or Nva;

A²² is Asn, Ala, Gln, Gly, N-Me-Asn, Aib, Anb, or Nva;

10 A²³ is Phe, any aromatic amino acid with or without substitutions, Leu, Ile, Val, Aib, Anb, Ala, or N-Me-Leu; and

Z is NHR₃ or OR₃; wherein R₃ is H, C₁-C₁₂ alkyl, C₇-C₁₀ phenylalkyl, C₃-C₂₀ alkenyl, C₃-C₂₀ alkynyl, phenyl, or naphthyl.

15 In one highly preferred embodiment, the amylin analog corresponds to amino acids 1 through 23 of human amylin with an amidated carboxy at the C-terminus, referred to herein as human amylin (1-23)-NH₂, having the following formula:

20 Lys-Cys-Asn-Thr-Ala-Thr-Cys-Ala-Thr-Gln-Arg-Leu-Ala-Asn-Phe-Leu-Val-His-Ser-Ser-Asn-Asn-Phe-NH₂. (SEQ ID NO:3)

In another highly preferred embodiment, the amylin analog corresponds to amino acids 1 through 23 of rat amylin, with an amidated carboxy at the C-terminus, referred to
25 herein as rat amylin (1-23)-NH₂, having the following formula:

Lys-Cys-Asn-Thr-Ala-Thr-Cys-Ala-Thr-Gln-Arg-Leu-Ala-Asn-Phe-Leu-Val-Arg-Ser-Ser-Asn-Asn-Leu-NH₂. (SEQ ID NO:4)

In yet another highly preferred embodiment, the amylin
30 analog corresponds to the derivative of amino acids 1 through 23 of rat amylin with α -amino normal butyric acid substitutions at positions 2 and 7, and an amidated

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carboxy at the C-terminus, referred to herein as [Anb^{2,7}]
rat amylin (1-23)-NH₂, having the following formula:

Lys-Anb-Asn-Thr-Ala-Thr-Anb-Ala-Thr-Gln-Arg-Leu-Ala-Asn-
Phe-Leu-Val-Arg-Ser-Ser-Asn-Asn-Leu-NH₂. (SEQ ID NO:5)

5 In another aspect, the invention features a method
of treating Type II diabetes mellitus in a human being by
administering a therapeutic amount of an amylin
antagonist of the invention. In a highly preferred
method of treatment of Type II diabetes mellitus, N- α -ac-
10 human amylin (8-23)-NH₂ is administered.

In another aspect, the invention features a method
of treating Type I diabetes mellitus in a human being by
administering a therapeutic amount of an amylin agonist
of the invention in conjunction with a therapeutic amount
15 of insulin.

In still another aspect, the invention features a
method of treating hypercalcemia by administering a
therapeutic amount of an amylin agonist of the invention.

The compounds of the invention exhibit a broad
20 range of biological activities, including those related
to glucose metabolism, calcium levels in the blood, and
appetite. Amylin antagonists of the invention attenuate
the inhibition by amylin of insulin-stimulated glucose
uptake. As a result, the amylin antagonists of the
25 invention act to reduce hyperglycemia resulting from
elevated levels of amylin associated with Type II
diabetes mellitus. Amylin agonists of the invention
inhibit insulin stimulated glucose uptake, thereby
tending to increase blood sugar levels. As a result, the
30 amylin agonists of the invention are useful in reducing
the hypoglycemia which frequently accompanies insulin
treatment of Type I diabetes mellitus. Amylin agonists
of the invention inhibit insulin stimulated glucose

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uptake, thereby tending to increase blood sugar levels. As a result, the amylin agonists of the invention are useful in reducing the hypoglycemia which frequently accompanies insulin treatment of Type I diabetes mellitus. Amylin agonists of the invention also decrease serum calcium levels, and are therefore useful for treating hypercalcemia. In addition, amylin agonists exhibit an appetite suppressant effect, while amylin antagonists increase appetite. Amylin agonists and antagonists are therefore useful in controlling food intake. For example, amylin agonists are useful for treating problems of overweight.

Many of the compounds of the invention are especially advantageous because they are truncated versions of the natural amylin peptide. The shorter peptide not only facilitates easier synthesis and purification of the compounds, but also improves selectivity and reduces manufacturing procedures and expenses.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

Detailed Description

The drawings will first be briefly described.

25 Drawings

Fig. 1 shows the comparison of the primary structures of human amylin (hAMYLIN) and rat amylin (rAMYLIN).

Fig. 2 shows the effect of human amylin, and N- α -ac-human amylin (8-23)-NH₂, separately and together, on glucose uptake in C₂C₁₂ muscle cells.

Fig. 3a and Fig. 3b show the in vivo effects of saline, rat amylin, N- α -ac-human amylin (8-23)-NH₂, and N- α -ac-human amylin (8-23)-NH₂ plus rat amylin on plasma

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glucose levels, and plasma insulin levels, respectively, in Sprague Dawley rats.

Fig. 4 shows the in vitro effect of human amylin and human amylin (1-23)-NH₂, separately, on insulin
5 stimulated glucose uptake in C₂C₁₂ muscle cells.

Fig. 5a and 5b show the in vivo effects of saline, rat amylin, human amylin (1-23)-NH₂, and human amylin (1-23)-NH₂ plus rat amylin on plasma glucose levels, and plasma insulin levels, respectively, in Sprague Dawley
10 rats.

Fig. 6 shows the in vitro effects of rat amylin (1-23)-NH₂ and [Anb^{2,7}] rat amylin (1-23)-NH₂, separately, on insulin stimulated glucose uptake in C₂C₁₂ muscle cells.

Fig. 7a and 7b show the in vivo effects of saline, rat amylin, [Anb^{2,7}] rat amylin (1-23)-NH₂, and [Anb^{2,7}] rat amylin (1-23)-NH₂ plus rat amylin on plasma glucose levels, and plasma insulin levels, respectively, in
15 Sprague Dawley rats.

20 Structure

The sequences of naturally occurring human amylin ("hAmylin") and rat amylin ("rAmylin") are set forth in Fig. 1. Balasubramaniam et al., Peptides, 12:919-924 (1991). There is a high degree of sequence homology
25 between amylin from these two species. It is believed that in naturally occurring hAmylin and rAmylin, the cysteine residues at positions 2 and 7, present in both species, form an internal disulfide bond, resulting in a cyclic structure.

30 The amylin analogs of the invention are based upon the biologically active subfragments comprising amino acids 8-23 of hAmylin and rAmylin and derivatives thereof; and upon the biologically active subfragments comprising amino acids 1-23 of hAmylin and rAmylin and

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derivatives thereof. In the amylin analog formulas set forth herein, the symbols A^x and the like; and Ser, Leu and the like, as found in a peptide sequence herein, stand for amino acid residues. When an amino acid residue is optically active, it is the L-form configuration that is intended unless the D-form is expressly designated. All peptide sequences mentioned herein are written according to the usual convention whereby the N-terminal amino acid is on the left and the C-terminal amino acid is on the right. A short line between two amino acid residues indicates a peptide bond. An -OR or an -NHR substituent on the carboxy terminal end of a peptide replaces the -OH on the carboxy terminal amino acid residue, yielding -NH-CH(R)-COOR, and -NH-CH(R)-CONHR as the C-terminal residues, respectively. When the carboxy terminal substituent is -NH₂, the peptide is in the amidated carboxy form.

As set forth above and for convenience in describing this invention, the conventional and nonconventional abbreviations for the various amino acids are used. They are familiar to those skilled in the art; but for clarity are listed below.

Asp =	D	=	Aspartic Acid
Ala =	A	=	Alanine
25 Arg =	R	=	Arginine
Asn =	N	=	Asparagine
Cys =	C	=	Cysteine
Gly =	G	=	Glycine
Glu =	E	=	Glutamic Acid
30 Gln =	Q	=	Glutamine
His =	H	=	Histidine
Ile =	I	=	Isoleucine
Leu =	L	=	Leucine
Lys =	K	=	Lysine

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Met = M = Methionine
Phe = F = Phenylalanine
Pro = P = Proline
Ser = S = Serine
5 Thr = T = Threonine
Trp = W = Tryptophan
Tyr = Y = Tyrosine
Val = V = Valine
Orn = Ornithine
10 Nal = 2-naphthylalanine
Nva = norvaline
Thi = 2-thienylalanine
Pcp = 4-chlorophenylalanine
Bth = 3-benzothienylalanine
15 Bip = 4,4'-biphenylalanine
Tic = tetrahydroisoquinoline-3-carboxylic acid
Aib = aminoisobutyric acid
Anb = α -aminonormalbutyric acid
Dip = 2,2-diphenylalanine

20 The compounds of the present invention can be provided in the form of pharmaceutically acceptable salts. Examples of preferred salts are those with therapeutically acceptable organic acids, e.g., acetic, lactic, maleic, citric, malic, ascorbic, succinic,
25 benzoic, salicylic, methane sulfonic, toluene sulfonic, trifluoroacetic, or pamoic acid, as well as polymeric acids such as tannic acid or carboxymethyl cellulose, and salts with inorganic acids, such as the hydrohalic acids, e.g., hydrochloric acid, sulfuric acid, or phosphoric
30 acid and the like.

Analysis

The structure-activity relationships of amylin and amylin analogs were studied both in an in vitro model using a mouse muscle cell line, C₂C₁₂, and an in vivo
35 model using Sprague Dawley rats.

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In the in vitro studies, insulin stimulated the glucose uptake by the C₂C₁₂ cell line in a dose-dependent manner and this was attenuated by rat amylin (100 pM). However, rat amylin did not exhibit any effect on the basal glucose uptake by this cell line. Cholera toxin did not have any effect on insulin stimulated glucose uptake but blocked the inhibitory effect of rat amylin.

Several partial sequences of human and rat amylin and their analogs were synthesized and their effects investigated in the in vitro and in vivo models.

Peptide Synthesis

Human and rat amylin were synthesized according to the procedures set forth in Balasubramaniam et al., Peptides, 12:919-924 (1991). The synthetic peptides were characterized by sequence and mass spectral analyses, and were found to be greater than 97% pure by analytical reversed-phase chromatography.

Peptide synthesis was accomplished on an Applied Biosystem Model 430A synthesizer. HPLC was carried out on a Waters Model 600 solvent delivery system in conjunction with a U6K injector, Model 481 spectrophotometer and Baseline 810 Data collection software in an IBM-XT computer. Protected amino acid derivatives (Peninsula, CA), synthesis reagents (Applied Biosystems, CA) and solvents (Fischer Scientific, OH) were obtained commercially and used without further purification. Paramethylbenzhydroxylamine (MBHA) resin (0.45 mmol, NH₂ group) was placed in the reaction vessel of the synthesizer and the amino acid derivatives were coupled automatically using the standard program provided by the manufacturers modified to incorporate a double

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coupling procedure. All amino acids were coupled using 2.2 equivalents of preformed symmetrical anhydrides. Arg, Asn, and Gln, however, were coupled as preformed 1-hydroxybenzotriazole esters (4.4 equivalent) to avoid deamidations or lactam formation. At the end of the synthesis the N- α -Boc group was removed, and the peptide resin (1.3 g) was treated with hydrogen fluoride (~10/ml) containing dimethylsulfide (~0.8 ml), p-cresol (~0.8 g) and p-thiocresol (~0.2 g) for one hour at -2 to 4°C. HF was evacuated and the residue transferred to a fritted filter funnel with diethyl ether, washed repeatedly with diethyl ether, extracted with acetic acid (2X15 ml) and lyophilized. The crude peptide (100 mg) thus obtained was dissolved in 6 M guanidine HCl (6 ml), diluted with 500 ml of distilled water and the Ph adjusted to 8 with ammonia. A solution of 0.1% potassium ferricyanide (w/v) was then added gradually with constant stirring until a permanent yellow color persisted. After stirring for an additional 30 minutes, the Ph of the solution was adjusted to 5 with acetic acid. The solution was then stirred with anion-exchange resin (AG-3, Cl⁻ form, 10 g wet weight) for 30 minutes, filtered through 0.45 microns filter and pumped into a semipreparative reversed phase column and purified as described in Balasubramaniam et al., Peptides, 12:919-924 (1991). The overall yield of rat and human amylin thus obtained varied between 10-20%.

In Vitro Assays

C₂C₁₂ cells were cultured at 37°C in a humidified 5% CO₂ atmosphere, in low glucose (1 g/l) DMEM medium containing 20% fetal bovine serum, and 0.5% chick embryo extract (growth medium). Cells were seeded in 75-cm² flasks at a density of 1x10⁶ cells per flask. When the cells became confluent (3-4 days), they were trypsinized (0.25% trypsin) and washed with growth medium. The final

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cell pellet was suspended in growth medium and seeded at a density of 2.5×10^4 cells/well into 24 well plates (16 mm diameter) and allowed to grow to 70% confluence (3 days). To induce fusion, the mononucleated myoblasts were exposed to medium containing 10% horse serum instead of 20% FBS (fusion medium). Fusion media was changed every day to avoid the premature detachment of cells and the cells were almost completely fused into multinucleated myotubes by the 9th day (6 days in fusion medium). Medium was changed one day before the experiment.

2-deoxyglucose uptake in C_2C_{12} myotubes was determined as described in Klip et al., Biochem. J., 242:131-136 (1987). In brief, cells were washed with PBS (phosphate-buffered saline) and incubated for 5h in the serum-free, high-glucose (25 mM) DMEM medium. At the end of incubation, cells were washed with PBS and different doses of amylin or amylin analogs (10pM- 10 μ M) were added and incubated with 2-deoxy-[3 H]-glucose (1mM) for 10 min. Non-carrier-mediated uptake was determined by incubating the cells with cytochalasin B (15 μ M). Uptake was terminated by rapidly aspirating the solution, and cells were washed with ice-cold PBS. Cell-associated radioactivity was determined by lysing the cells in 1 M NaOH and the aliquots were neutralized and counted in a scintillation counter. Protein content of the aliquots was determined by the Lowry method.

After seeding, the undifferentiated mononucleated myoblasts grew logarithmically and reached 70% confluence by day 3. Fused cells were detected by day 5 and contained >90% multinucleated myotubes by day 9 (6 days in fusion media). In 6-day-old cells there was a 30% increase in glucose uptake in response to insulin compared to a 68-115% increase in 9-day-old cells. These results are similar to earlier observations (Klip et al.,

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supra). The low insulin response by 6-day-old cells, presumably, is due to the presence of undifferentiated myoblasts with low insulin-receptor density as evident in the L₆ muscle cell line (Beguinot et al., Endocrinology, 5 18:446-455 (1986)). Because of these findings, and the observation that insulin stimulated glucose uptake in 9-day-old C₂C₁₂ cells to test the effects of amylin or amylin analogs on insulin-stimulated glucose uptake. The 10 maximal insulin-stimulated response was observed at 100 nM and remained plateaued at further increasing doses. The insulin-stimulated glucose uptake in C₂C₁₂ myotubes appears to occur mainly through facilitated diffusion because cytochalasin B (15 μ M) inhibited >90% of insulin- 15 stimulated 2-deoxyglucose uptake by the cells.

In Vivo Assays

Sprague Dawley rats (Zivic Miller, Zelienople, PA) used in this investigation were housed individually in air-conditioned rooms (22-24°C) under 12-hour light/dark 20 cycle with ad lib access to Purina rat chow and water.

Sprague Dawley rats weighing about 300g were fasted overnight (18-22 hrs). Rats were then anesthetized with sodium pentobarbital (40 mg/kg) and catheters were implanted in the jugular vein. Saline 25 (0.1 ml), rat amylin (50 μ g) in saline (0.1ml) or peptide fragments/analogues (100 μ g) in saline (0.1 ml) were injected through the jugular vein and then flushed with another 0.1 ml of saline. In the cases of studying antagonistic effects, injection of peptide 30 fragments/analogues (100 μ g) in saline (0.1 ml) were followed 2 min. later with rat amylin (50 μ g) in saline (0.1 ml) injection. 30 min. after the injection of the peptides, blood (4-5 ml) was drawn through the jugular vein and collected in heparinized tubes containing 35 aprotinin (10 μ l). Plasma was obtained by

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centrifugation. Plasma glucose and insulin levels were determined by the glucose oxidase method (Model 27 glucose analyzer, Yellow Springs Instruments, Yellow Springs, OH) and a radioimmunoassay kit (Peninsula Laboratories, Belmont, CA), respectively.

Results

Referring to Fig. 2, one of the antagonists of the invention, N- α -ac-human amylin(8-23)-NH₂, exhibited no significant effect on insulin stimulated glucose uptake in the in vitro assay when tested separately. Still referring to Fig. 2, the presence of N- α -ac-human amylin (8-23)-NH₂ (1 μ M) with human amylin consistently shifted the inhibitory dose-response curve of human amylin on insulin stimulated glucose uptake to the right (*i.e.*, higher concentrations of human amylin), increasing the IC₅₀ value from 0.20 nM to 350 nM.

In vivo effects of N- α -ac-human amylin (8-23)-NH₂ were investigated in anesthetized (45 mg/kg) Sprague Dawley rats (~300 g) fasted overnight (\geq 20 h). The following samples were injected via a cannulated jugular vein into individual rats: (1) 100 μ l of saline (n = 5), (2) rat amylin (50 μ g), (3) N- α -ac-human amylin (8-23)-NH₂ (100 μ g), and (4) N- α -ac-human amylin (8-23)-NH₂ (100 μ g) followed 2 min later by rat amylin (50 μ g). Thirty minutes after injection, 4-5 ml blood was collected in heparinized tubes from each of the rats and the plasma separated by centrifugation. Plasma glucose and insulin levels were subsequently determined, and the results are set forth in Fig. 3a and 3b, respectively.

Referring to Fig. 3a, rat amylin significantly increased the plasma glucose level compared to the saline control, while N- α -ac-human amylin (8-23)-NH₂ significantly decreased the plasma glucose levels relative to the control, probably by antagonizing the

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effects of endogenous amylin. Still referring to Fig. 3a, N- α -ac-human amylin (8-23)-NH₂ significantly attenuated the elevation of plasma glucose by rat amylin in the rat which received both N- α -ac-human amylin (8-23)-NH₂ and rat amylin (i.e. plasma glucose levels were brought down near the control value). The p values in Fig. 3a and 3b, and throughout, refer to values obtained using the ANOVA program with n equal to 5 to 8.

These observations confirm that N- α -ac-human amylin (8-23)-NH₂ is a potent antagonist of human amylin in vitro, and of rat amylin in vivo.

Referring to Fig. 4, human amylin (1-23)-NH₂ inhibited insulin stimulated glucose uptake in the in vitro assay in a manner similar to human amylin. Still referring to Fig. 4, human amylin (1-23)-NH₂ exhibited a dose-response inhibitory effect on insulin-stimulated glucose uptake by C₂C₁₂ cells with a potency comparable to that of intact human amylin.

Referring to Fig. 5a, human amylin (1-23)-NH₂ attenuated rat amylin induced hyperglycemia.

Referring to Fig. 6, [Anb^{2,7}] rat amylin(1-23)-NH₂ inhibited the insulin stimulated glucose uptake in the in vitro assay in a manner qualitatively similar to rat amylin(1-23)-NH₂. Referring to Fig. 6 and Fig. 4, rat amylin (1-23)-NH₂ exhibited a dose-response inhibitory effect on insulin-stimulated glucose uptake by C₂C₁₂ cells with a potency comparable to that of intact human amylin. Still referring to Fig. 6 and Fig. 4, [Anb^{2,7}] rat amylin (1-23)-NH₂ also exhibited a potency comparable to that of human amylin.

Referring to Fig. 7, [Anb^{2,7}] rat amylin(1-23)-NH₂ had no significant effect on amylin induced hyperglycemia, but the tendency was in the direction of attenuation.

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The results obtained together with reported data in the literature are set forth in Table 1 below.

PEPTIDES	C ₂ C ₁₂ -effect on		ANESTHETIZED RATS	
	insulin stimulated glucose uptake ¹		PLASMA GLUCOSE ^{1,2}	plasma Ca ²⁺
1. human amylin ("HA")	inhibits (Fig. 2)		elevates ³	lowered ⁴
2. rat amylin ("RA")	inhibits ⁵		elevates ³	lowered ⁴
3. HA(1-23)-NH ₂	inhibits (similar to human amylin) (Fig. 4)		1. lowers basal (Fig. 5a)	N.D.
			2. attenuates amylin induced hyperglycemia	
4. RA(1-23)-NH ₂	inhibits (similar to human amylin) (Fig. 6)		N.D.	N.D.
5. [Anb ^{2,7}]RA(1-23)-NH ₂	inhibits (similar to human amylin) (Fig. 6)		1. lowers basal (Fig. 7a)	N.D.
			2. no effect on amylin induced hyperglycemia	
6. N-α-Ac-HA((8-23)-NH ₂	1. no effect		1. lowers basal (Fig. 3a)	N.D.
	2. attenuates amylin effects (Fig. 2)		2. attenuates amylin induced hyperglycemia	

1. Present study; 2. effects of 100 µg peptide analogs on basal or 50 µg rat amylin induced hyperglycemia; 3. Molina et al., Diabetes, 39:260-265 (1990); and Young et al., Am. J. Physiology, 259: E457-461 (1990); 4. Data et al., Biochem. Biophys. Res. Commun., 162:876-881 (1989); 5. Sherliff et al., Biochim. Biophys. Acta, 1136: 219-222 (1992).

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The agonist or antagonist effect of other amylin analogs of the invention may be determined by the assays described above.

USE

Amylin inhibits insulin stimulated glucose uptake and glycogen synthesis, and increases the hepatic glucose output. Therefore, it appears that a particular ratio of insulin to amylin is required to maintain the normal plasma glucose levels.

The amylin agonists and antagonists of the invention have useful applications in treating Type I and II diabetes mellitus, respectively. Since humans with Type II diabetes mellitus have elevated levels of amylin and elevated blood glucose levels, administration of an amylin antagonist of the invention in an amount sufficient to decrease blood glucose levels to normal or clinically acceptable levels provides therapeutic results. Humans with Type I diabetes mellitus have decreased levels of both insulin and amylin, and when treated with insulin have a tendency to develop hypoglycemia. Administration of an amylin agonist of the invention in an amount sufficient to increase blood glucose levels to normal or clinically acceptable levels in response to insulin induced hypoglycemia, together with a therapeutic amount of insulin, provides therapeutic results.

Amylin agonists of the invention decrease serum calcium levels and may be administered to humans to treat hypercalcemia. Amylin agonists of the invention exhibit an appetite suppressant effect, while amylin antagonists increase appetite. Amylin agonists and antagonists of the invention are therefore useful in controlling food intake. For example, amylin agonists of the invention may be administered for the treatment of obesity.

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The peptides of the invention may be administered to a human in one of the traditional modes (e.g., orally, parenterally, transdermally, or transmucosally), or in a sustained release formulation using a biodegradable biocompatible polymer.

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SEQUENCE LISTING**(1) GENERAL INFORMATION:**

(i) APPLICANT: A. Balasubramaniam
(ii) TITLE OF INVENTION: AMYLIN ANTAGONISTS AND AGONISTS
(iii) NUMBER OF SEQUENCES: 7
(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Fish & Richardson
(B) STREET: 225 Franklin Street
(C) CITY: Boston
(D) STATE: Massachusetts
(E) COUNTRY: U.S.A.
(F) ZIP: 02110-2804

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: 3.5" Diskette, 1.44 Mb
(B) COMPUTER: IBM PS/2 Model 50Z or 55SX
(C) OPERATING SYSTEM: MS-DOS (Version 5.0)
(D) SOFTWARE: WordPerfect (Version 5.1)

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: 08/060,265
(B) FILING DATE: 12 May 1993

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Clark, Paul T.
(B) REGISTRATION NUMBER: 30,162
(C) REFERENCE/DOCKET NUMBER: 00537/078WO1

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: (617) 542-5070
(B) TELEFAX: (617) 542-8906
(C) TELEX: 200154

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 1:**(i) SEQUENCE CHARACTERISTICS:**

(A) LENGTH: 16
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

N α Ac Ala Thr Gln Arg Leu Ala Asn Phe Leu Val His Ser Ser Asn Asn Phe NH₂
5 10 15

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 2:

(A) LENGTH: 16
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: Linear

N α Ac Ala Thr Gln Arg Leu Ala Asn Phe Leu Val Arg Ser Ser Asn Asn Leu NH₂
5 10 15

(A) LENGTH: 23
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: Linear

Lys Cys Asn Thr Ala Thr Cys Ala Thr Gln Arg Leu Ala Asn Phe Leu
5 10 15
Val His Ser Ser Asn Asn Phe NH₂
20

(A) LENGTH: 23
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: Linear

Lys Cys Asn Thr Ala Thr Cys Ala Thr Gln Arg Leu Ala Asn Phe Leu
5 10 15
Val Arg Ser Ser Asn Asn Leu NH₂
20

(A) LENGTH: 23
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: Linear

Lys Anb Asn Thr Ala Thr Anb Ala Thr Gln Arg Leu Ala Asn Phe Leu
5 10 15
Val Arg Ser Ser Asn Asn Leu NH₂
20

- 25 -

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 37
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Lys Cys Asn Thr Ala Thr Cys Ala Thr Gln Arg Leu Ala Asn Phe Leu
 5 10 15
 Val His Ser Ser Asn Asn Phe Gly Ala Ile Leu Ser Ser Thr Asn Val
 20 25 30
 Gly Ser Asn Thr Tyr
 35

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 37
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
 (D) TOPOLOGY: Linear

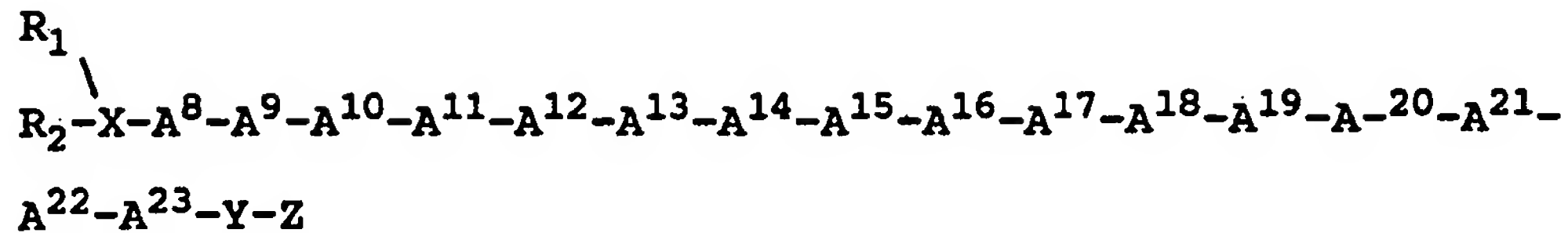
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Lys Cys Asn Thr Ala Thr Cys Ala Thr Gln Arg Leu Ala Asn Phe Leu
 5 10 15
 Val Arg Ser Ser Asn Asn Leu Gly Pro Val Leu Pro Pro Thr Asn Val
 20 25 30
 Gly Ser Asn Thr Tyr
 35

What is claimed is:

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1. An amylin analog of the amino acid formula:



wherein:

X is a chain of 0-5 amino acids, inclusive, the N-terminal one of which is bonded to R_1 and R_2 ;

Y is a chain of 0-4 amino acids, inclusive, the C-terminal one of which is bonded to Z;

Each of R_1 , and R_2 , independently, is H, C_1 - C_{12} alkyl (e.g., methyl), C_6 - C_{18} aryl (e.g., phenyl, naphthaleneacetyl), C_1 - C_{12} acyl (e.g., formyl, acetyl, and myristoyl), C_7 - C_{18} aralkyl (e.g., benzyl); or C_7 - C_{18} alkaryl (e.g., *p*-methylphenyl);

A^8 is Ala, Nal, Thi, Phe, Bth, Pcp, N-Me-Thr, Aib, or Anb;

A^9 is Thr, Ala, Anb, Aib, Ser, N-Me-Ser, or N-Me-Thr;

A^{10} is Gln, Ala, Asn, N-Me-Gln, Gly, Nva, Aib, or Anb;

A^{11} is Arg, homo-Arg, diethyl-homo-Arg, Lys- ϵ -NH-R (where R is H, a branched or straight chain C_1 - C_{10} alkyl group, or an aryl group), Orn, or Lys;

A^{12} is Leu, Ile, Val, Aib, Anb, or N-Me-Leu;

A^{13} is Ala, Nal, Thi, Phe, Bth, Pcp, N-Me-Thr, Aib, or Anb;

A^{14} is Asn, Ala, Gln, Gly, N-Me-Asn, Nva, Aib, or Anb;

A^{15} is Phe, or any aromatic amino acid with or without substituents;

A^{16} is Leu, Ile, Val, Aib, Anb, or N-Me-Leu;

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A¹⁷ is Val, Ile, Aib, Anb, or N-Me-Val;

A¹⁸ is His, Thr, 3-Me-His, 1-Me-His, β -pyrolylalanine, N-Me-His, Arg, homo-Arg, diethyl-homo-Arg, Lys- ϵ -NH-R (where R is H, a branched or straight chain C₁-C₁₀ alkyl group, or an aryl group), Ala, Aib, Anb, or Orn;

A¹⁹ is Ser, Thr, N-Me-Ser, N-Me-Thr, Aib, Anb, or Ala;

A²⁰ is Ser, Thr, N-Me-Ser, N-Me-Thr, Aib, Anb, or Ala;

A²¹ is Asn, Ala, Gln, Gly, N-Me-Asn, Aib, Anb, or Nva;

A²² is Asn, Ala, Gln, Gly, N-Me-Asn, Aib, Anb, or Nva;

A²³ is Phe, any aromatic amino acid with or without substituents, Leu, Ile, Val, Aib, Anb, Ala, or N-Me-Leu; and

Z is NHR₃ or OR₃; wherein R₃ is H, C₁-C₁₂ alkyl, C₇-C₁₀ phenylalkyl, C₃-C₂₀ alkenyl, C₃-C₂₀ alkynyl, phenyl, or naphthyl.

or a pharmaceutically acceptable salt thereof.

2. An amylin analog of claim 1 which is an antagonist.

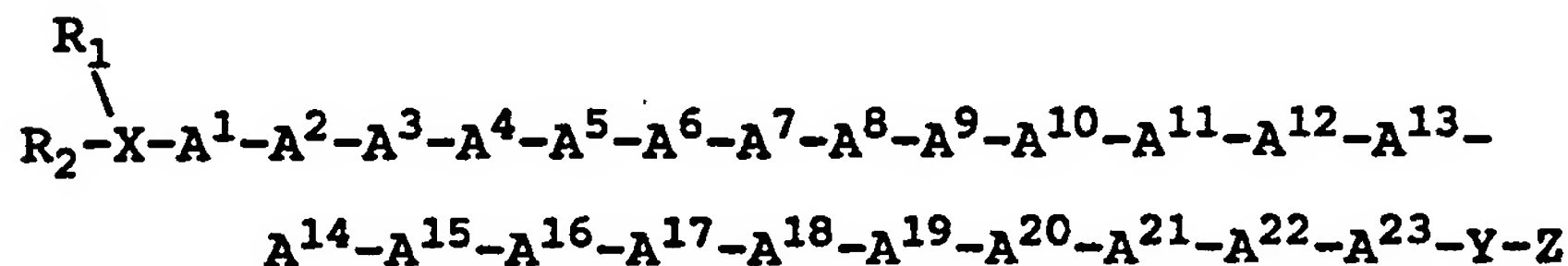
3. An amylin analog of claim 2 corresponding to the N- α -acetyl derivative of amino acids 8 through 23 of human amylin with an amidated carboxy at the C-terminus ("N- α -ac-human amylin (8-23)-NH₂") having the formula: N- α -ac-Ala-Thr-Gln-Arg-Leu-Ala-Asn-Phe-Leu-Val-His-Ser-Ser-Asn-Asn-Phe-NH₂, or a pharmaceutically acceptable salt thereof.

4. An amylin analog of claim 2 having the amino acid formula:

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N- α -Ac-Ala-Thr-Gln-Arg-Leu-Ala-Asn-Phe-Leu-Val-Arg-Ser-Ser-Asn-Asn-Leu-NH₂, or a pharmaceutically acceptable salt thereof.

5. An amylin analog of the amino acid formula:



wherein

X is a chain of 0-5 amino acids, inclusive, the N-terminal one of which is bonded to R₁ and R₂;

Y is a chain of 0-4 amino acids, inclusive, the C-terminal one of which is bonded to Z;

Each of R₁, and R₂, independently, is H, C₁-C₁₂ alkyl, C₆-C₁₈ aryl, C₁-C₁₂ alyl, C₇-C₁₈ aralkyl, or C₇-C₁₈ alkaryl;

A¹ is Lys, Arg, homo-Arg, diethyl-homo-Arg, Lys- ϵ -NH-R (where R is H, a branched or straight chain C₁-C₁₀ alkyl group, or an aryl group), or Orn;

A² is Cys, or Anb;

A³ is Asn, Ala, Gln, Gly, N-Me-Asn, Aib, Anb, or Nva;

A⁴ is Thr, Ser, N-Me-Ser, N-Me-Thr, Ala, Aib, or Anb;

A⁵ is Ala, Nal, Thi, Phe, Bth, Pcp, N-Me-Ala, Aib, or Anb;

A⁶ is Thr, Ser, N-Me-Ser, or N-Me-Thr, Ala, Aib, or Anb;

A⁷ is Cys, or Anb;

A⁸ is Ala, Nal, Thi, Phe, Bth, Pcp, N-Me-Ala, Aib, or Anb;

A⁹ is Thr, Ser, N-Me-Ser, N-Me-Thr, Ala, Aib, or Anb;

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A¹⁰ is Gln, Ala, Asn, N-Me-Gln, Gly, Nva, Aib, or Anb;

A¹¹ is Arg, homo-Arg, diethyl-homo-Arg, Lys- ϵ -NH-R (where R is H, a branched or straight chain C₁-C₁₀ alkyl group, or an aryl group), or Orn;

A¹² is Leu, Ile, Val, Aib, Anb, or N-Me-Leu;

A¹³ is Ala, Nal, Thi, Phe, Bth, Pcp, N-Me-Ala, Aib, or Anb;

A¹⁴ is Asn, Ala, Gln, Gly, N-Me-Asn, Aib, Anb, or Nva;

A¹⁵ is Phe, or any aromatic amino acid with or without substituents;

A¹⁶ is Leu, Ile, Val, Aib, Anb, or N-Me-Leu;

A¹⁷ is Val, Ile, Aib, Anb, or N-Me-Val;

A¹⁸ is His, Thr, 3-Me-His, 1-Me-His, β -pyroazolylalanine, N-Me-His, Arg, homo-Arg, diethyl-homo-Arg, Lys- ϵ -NH-R (where R is H, a branched or straight chain C₁-C₁₀ alkyl group, or an aryl group), Orn, Ala, Aib, or Anb;

A¹⁹ is Ser, Thr, N-Me-Ser, N-Me-Thr, Ala, Aib, or Anb;

A²⁰ is Ser, Thr, N-Me-Ser, N-Me-Thr, Ala, Aib, or Anb;

A²¹ is Asn, Ala, Gln, Gly, N-Me-Asn, Aib, Anb, or Nva;

A²² is Asn, Ala, Gln, Gly, N-Me-Asn, Aib, Anb, or Nva;

A²³ is Phe, any aromatic amino acid with or without substitutions, Leu, Ile, Val, Aib, Anb, Ala, or N-Me-Leu; and

Z is NHR₃ or OR₃; wherein R₃ is H, C₁-C₁₂ alkyl, C₇-C₁₀ phenylalkyl, C₃-C₂₀ alkenyl, C₃-C₂₀ alkynyl, phenyl, or naphthyl.

or a pharmaceutically acceptable salt thereof.

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6. An amylin analog of claim 5 corresponding to amino acids 1 through 23 of human amylin with an amidated carboxy at the C-terminus ("human amylin (1-23)-NH₂"), having the formula:

Lys-Cys-Asn-Thr-Ala-Thr-Cys-Ala-Thr-Gln-Arg-Leu-Ala-Asn-Phe-Leu-Val-His-Ser-Ser-Asn-Asn-Phe-NH₂, or a pharmaceutically acceptable salt thereof.

7. An amylin analog of claim 5 corresponding to amino acids 1 through 23 of rat amylin, with an amidated carboxy at the C-terminus ("rat amylin (1-23)-NH₂"), having the formula:

Lys-Cys-Asn-Thr-Ala-Thr-Cys-Ala-Thr-Gln-Arg-Leu-Ala-Asn-Phe-Leu-Val-Arg-Ser-Ser-Asn-Asn-Leu-NH₂, or a pharmaceutically acceptable salt thereof.

8. An amylin analog of claim 5 corresponding to the derivative of amino acids 1 through 23 of rat amylin with α -amino normal butyric acid substitutions at positions 2 and 7, and an amidated carboxy at the C-terminus ("[Anb^{2,7}] rat amylin (1-23)-NH₂") having the formula:

Lys-Anb-Asn-Thr-Ala-Thr-Anb-Ala-Thr-Gln-Arg-Leu-Ala-Asn-Phe-Leu-Val-Arg-Ser-Ser-Asn-Asn-Leu-NH₂, or a pharmaceutically acceptable salt thereof.

9. A method of treating Type II diabetes mellitus in a human being comprising administering to said human being a therapeutic amount of an amylin antagonist of claim 2.

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10. The method of claim 9 in which said amylin antagonist is N- α -ac-human amylin (8-23)-NH₂.

11. A method of treating Type I diabetes mellitus in a human being comprising administering to said human being a therapeutic amount of an amylin analog of claim 5 which is an agonist, and a therapeutic amount of insulin.

12. A method of treating hypercalcemia in a human being comprising administering to said human being a therapeutic amount of an amylin analog of claim 5 which is an agonist.

13. A method of controlling food intake in a human being comprising administering to said human being a therapeutic amount of an amylin analog of claim 1 or claim 5.

1 5 10 15
Lys Cys Asn Thr Ala Thr Cys Ala Thr Gln Arg Leu Ala Asn Phe
16 20 25 30
Leu Val His Ser Ser Asn Asn Phe Gly Ala Ile Leu Ser Ser Thr
31 35
Asn Val Gly Ser Asn Thr Tyr

hAMYLIN (SEQ ID NO:6)

1 5 10 15
Lys Cys Asn Thr Ala Thr Cys Ala Thr Gln Arg Leu Ala Asn Phe
16 20 25 30
Leu Val Arg Ser Ser Asn Asn Leu Gly Pro Val Leu Pro Pro Thr
31 35
Asn Val Gly Ser Asn Thr Tyr

rAMYLIN (SEQ ID NO:7)

FIG. 1

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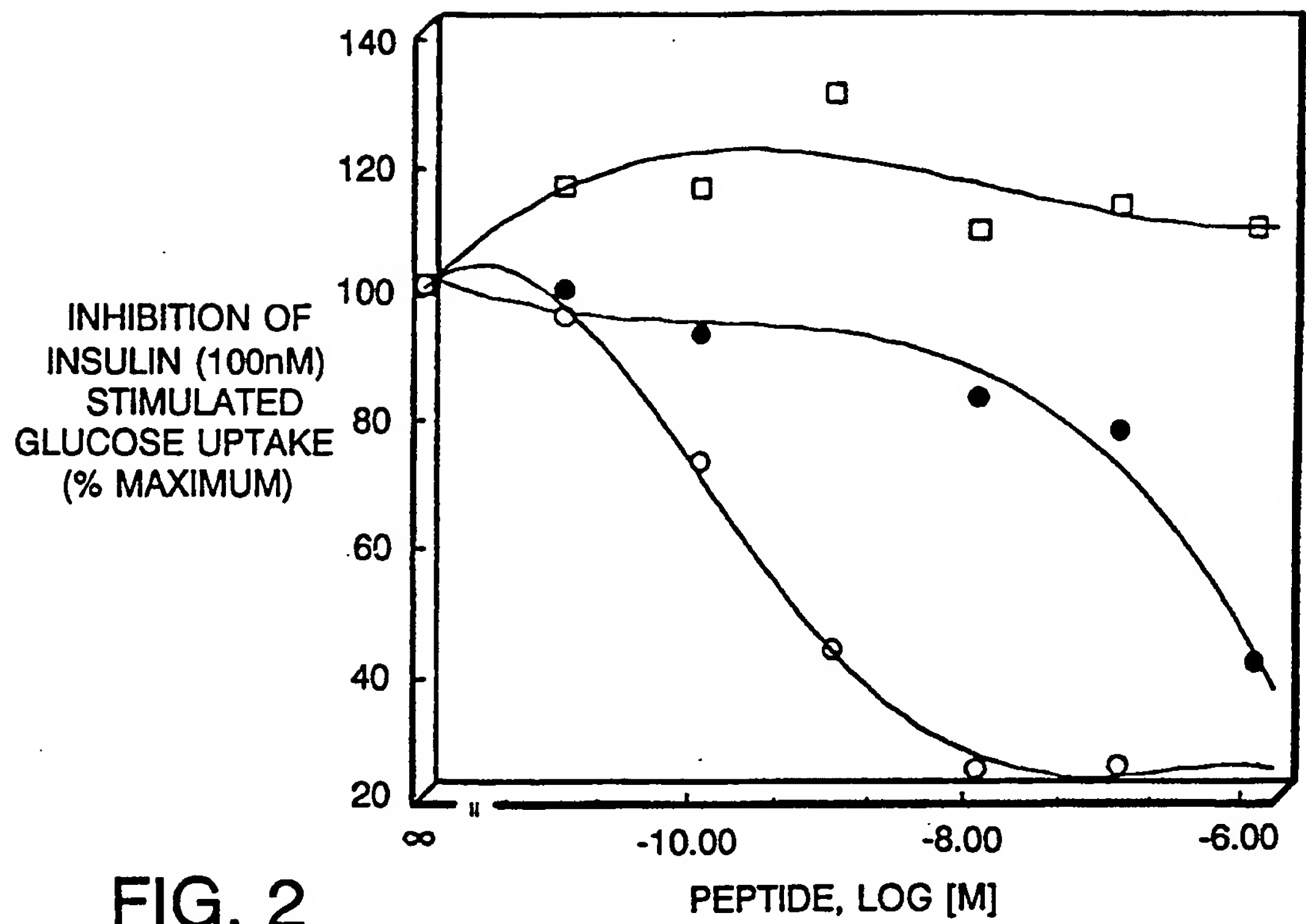


FIG. 2

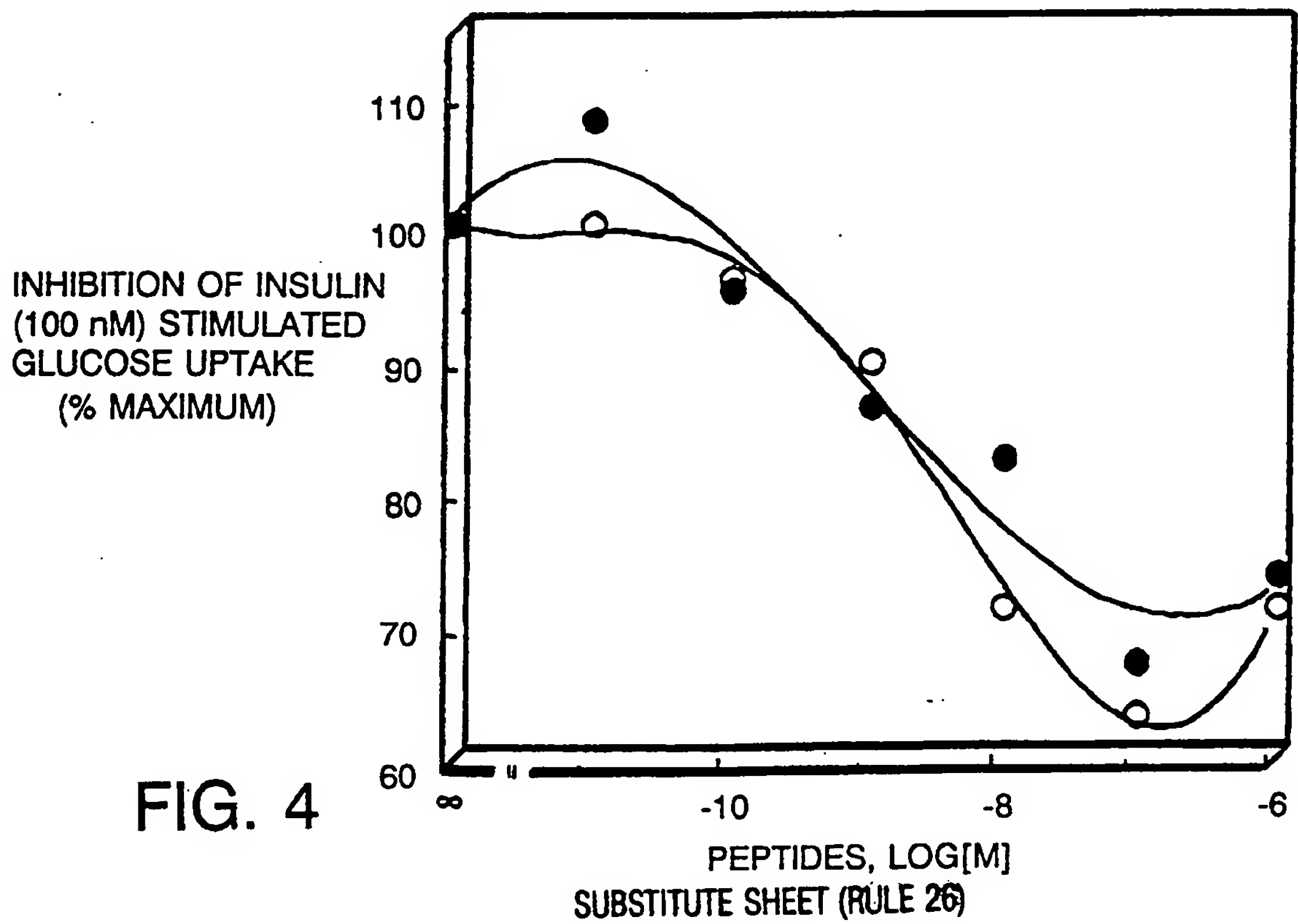


FIG. 4

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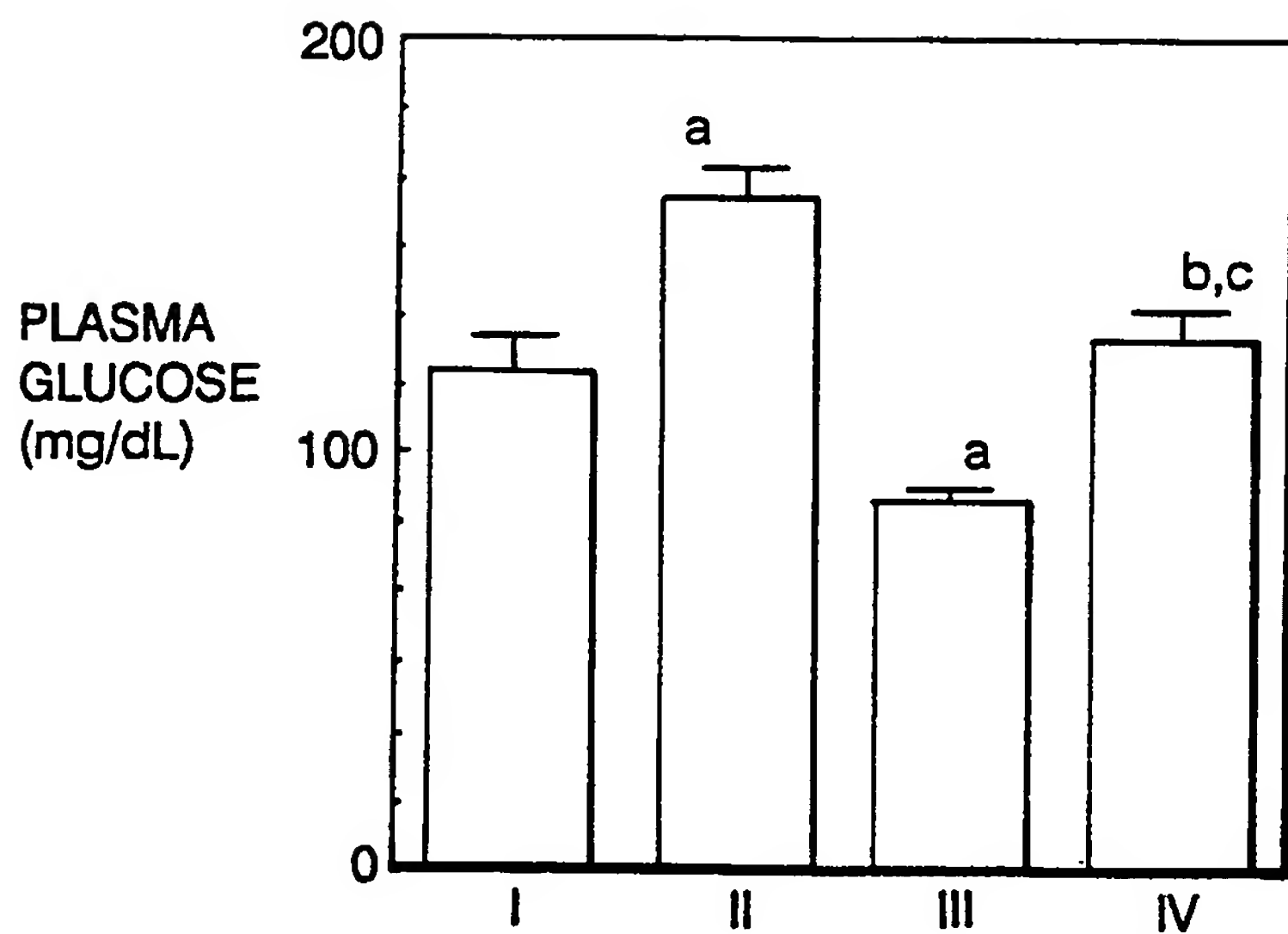


FIG. 3A

a = $p < 0.05$ vs control
b = not significant vs control
c = $p < 0.05$ vs rat amylin

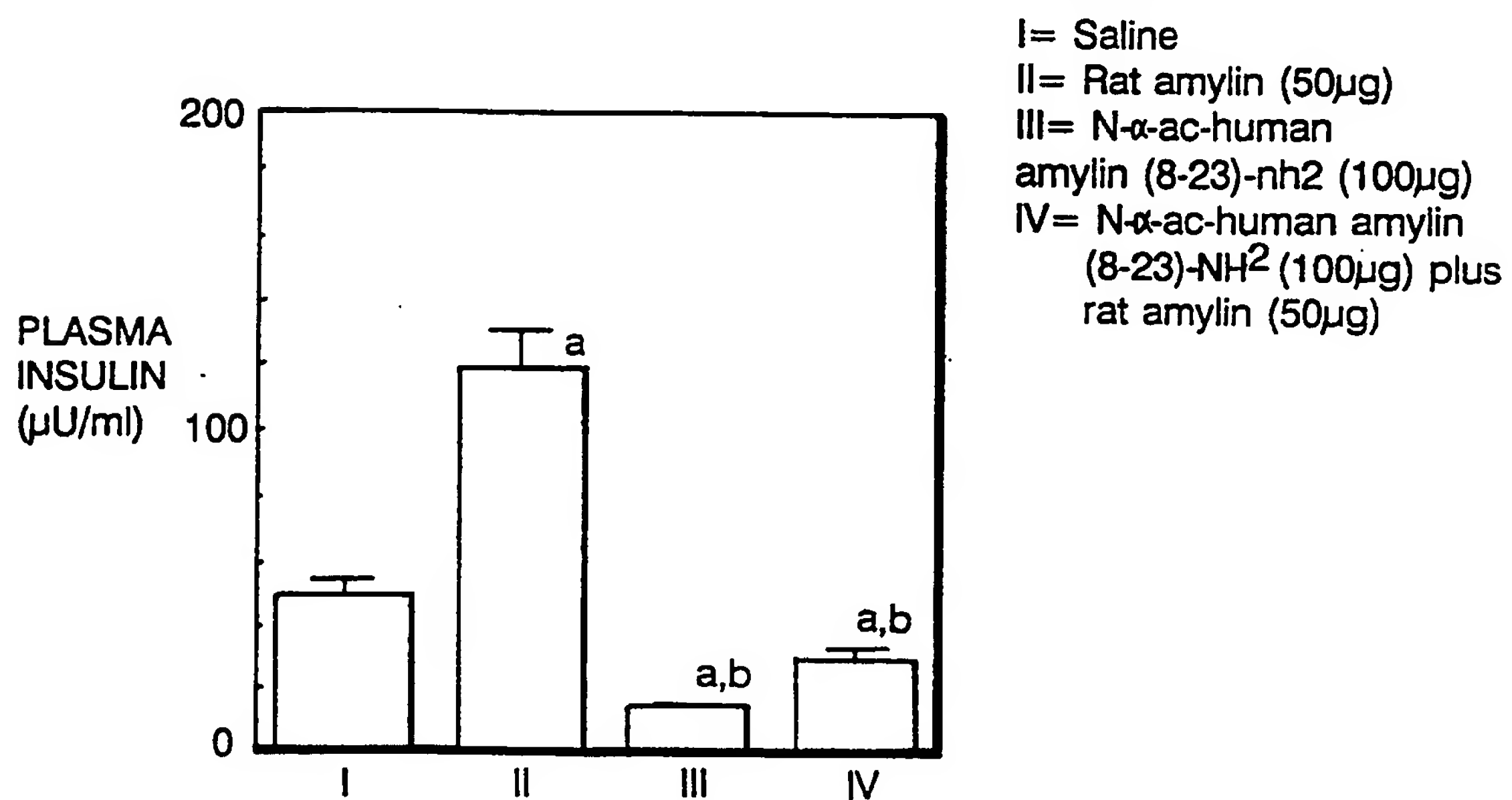


FIG. 3B

a = $p < 0.05$ vs control
b = $p < 0.05$ vs rat amylin

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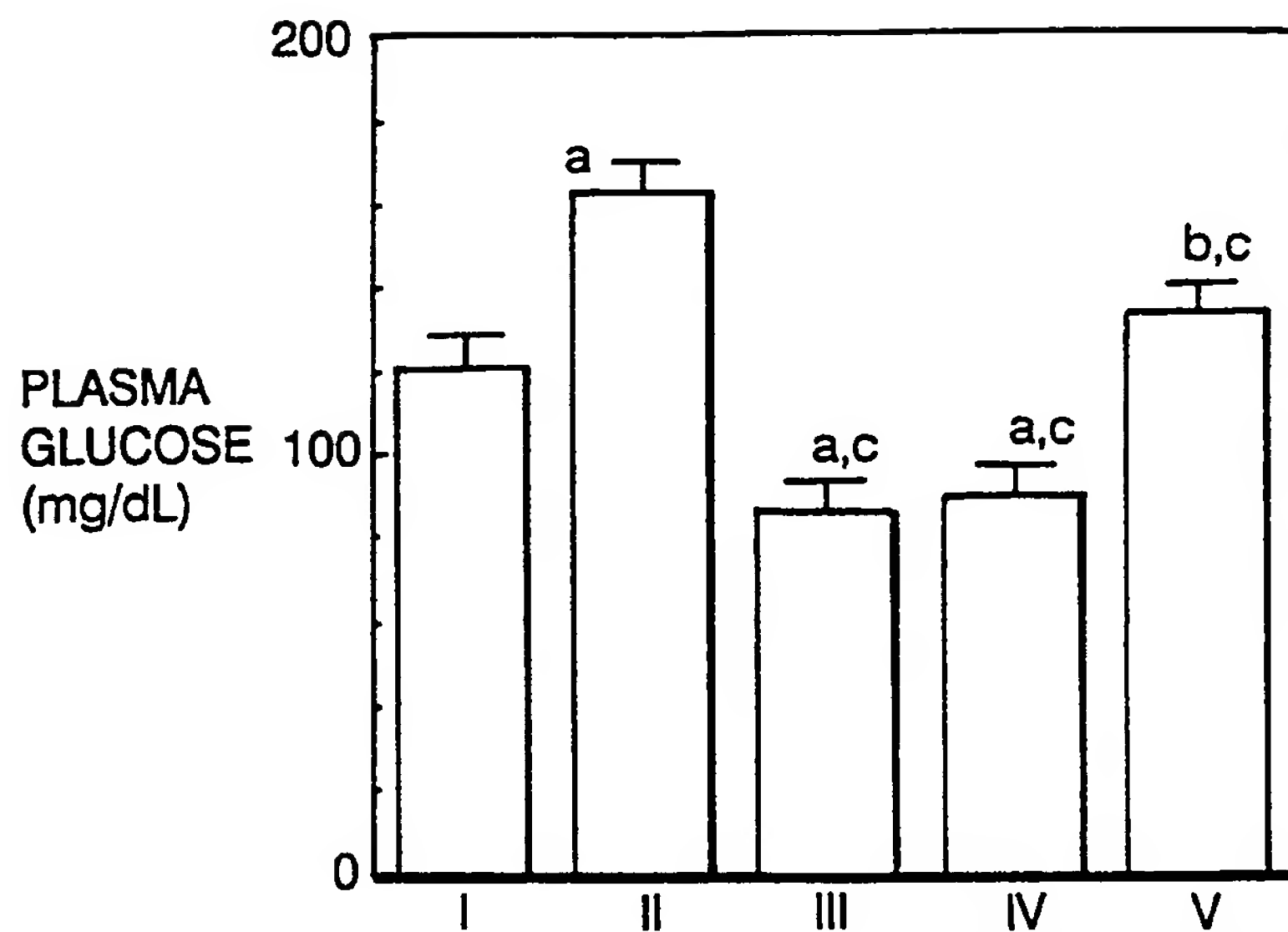


FIG. 5A

a = $p < 0.05$ vs control
 b = not significant vs control
 c = $p < 0.05$ vs rat amylin

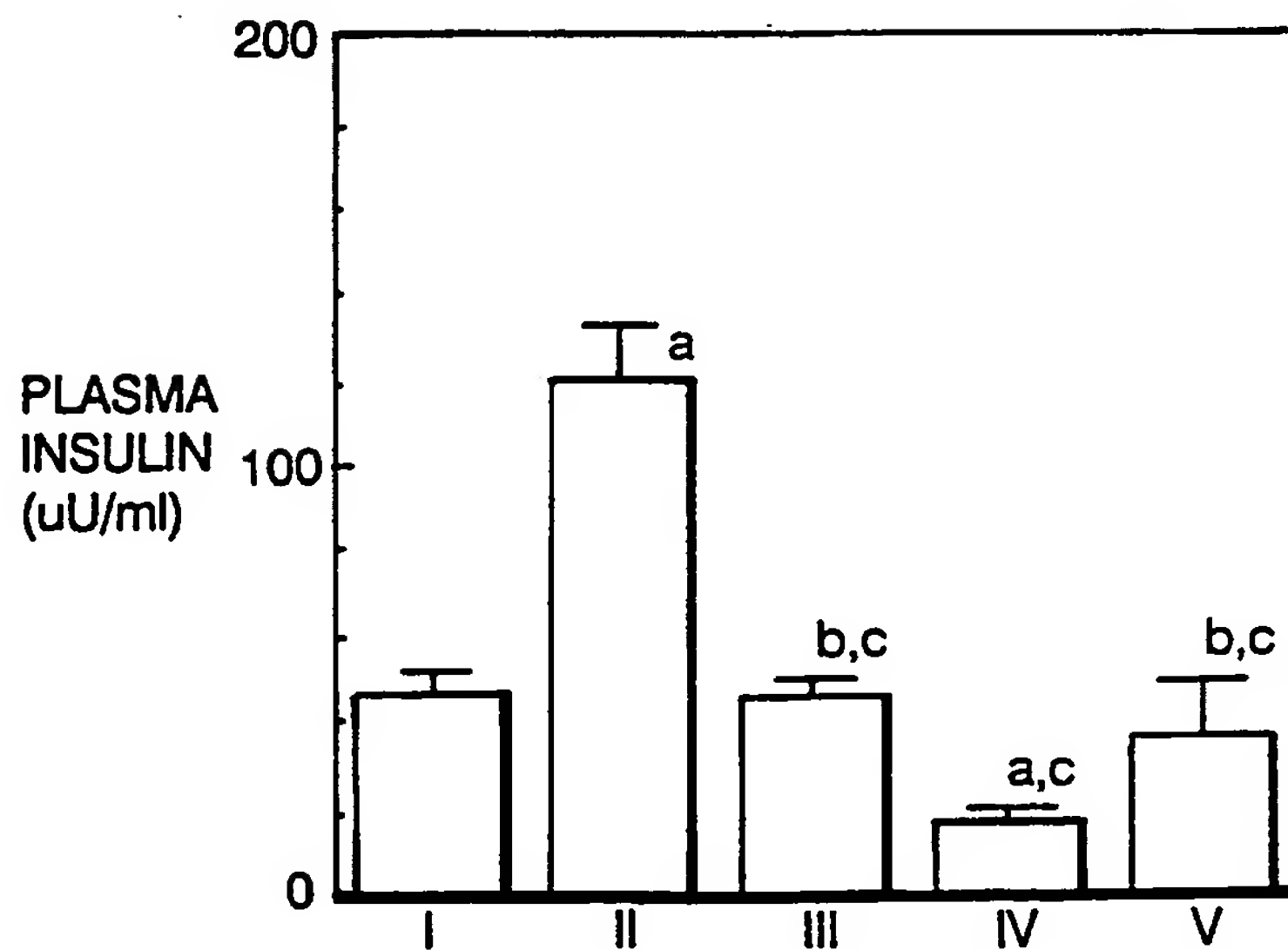


FIG. 5B

a = $p < 0.05$ vs control
 b = not significant vs control
 c = $p < 0.05$ vs rat amylin

I = Saline
 II = Rat amylin (50µg)
 III = Human amylin (1-23)-NH₂ (50µg)
 IV = Human amylin (1-23)-NH₂ (100µg)
 V = Human amylin (1-23)-NH₂ (100µg)
 plus rat amylin (50µg)

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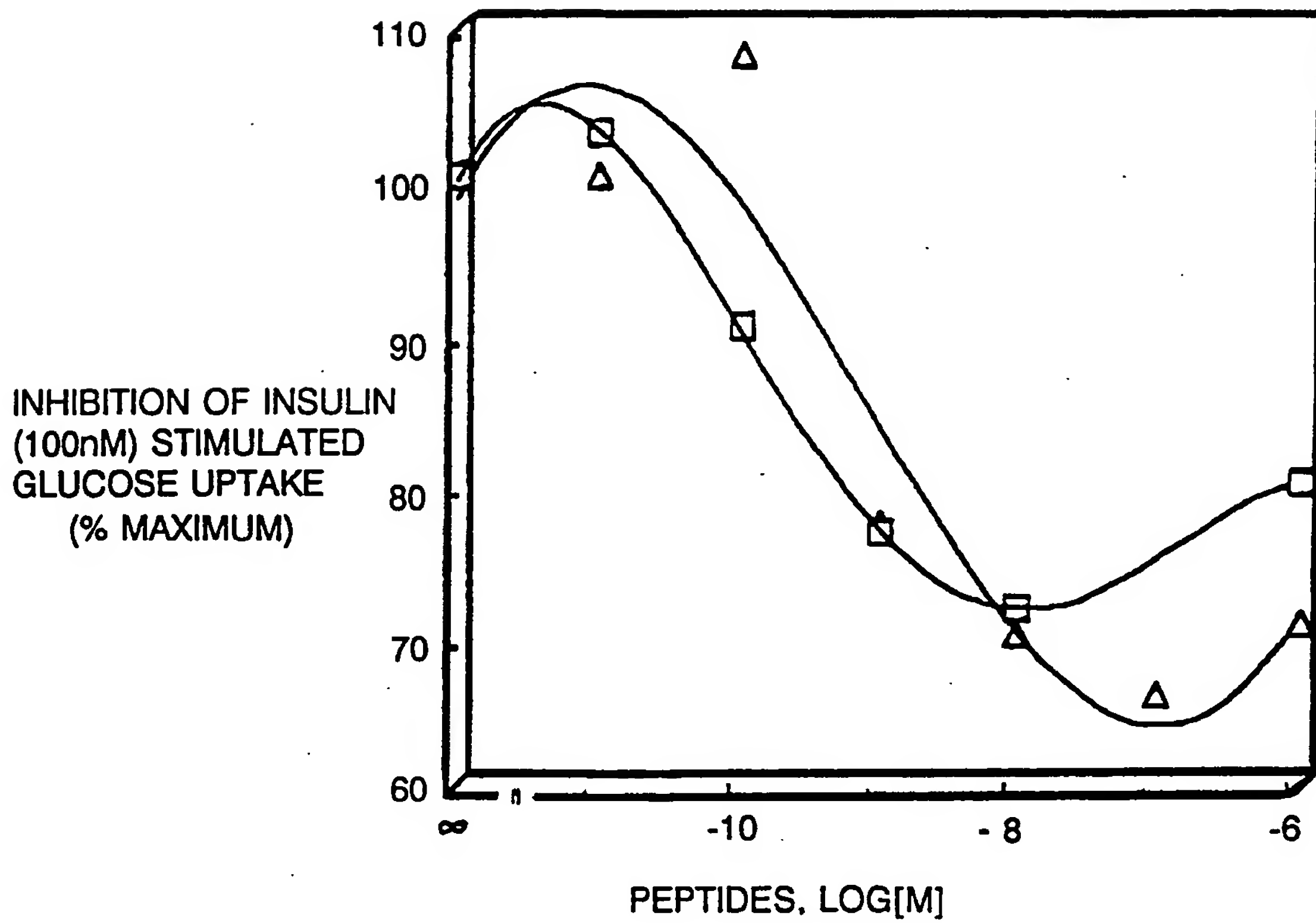


FIG. 6

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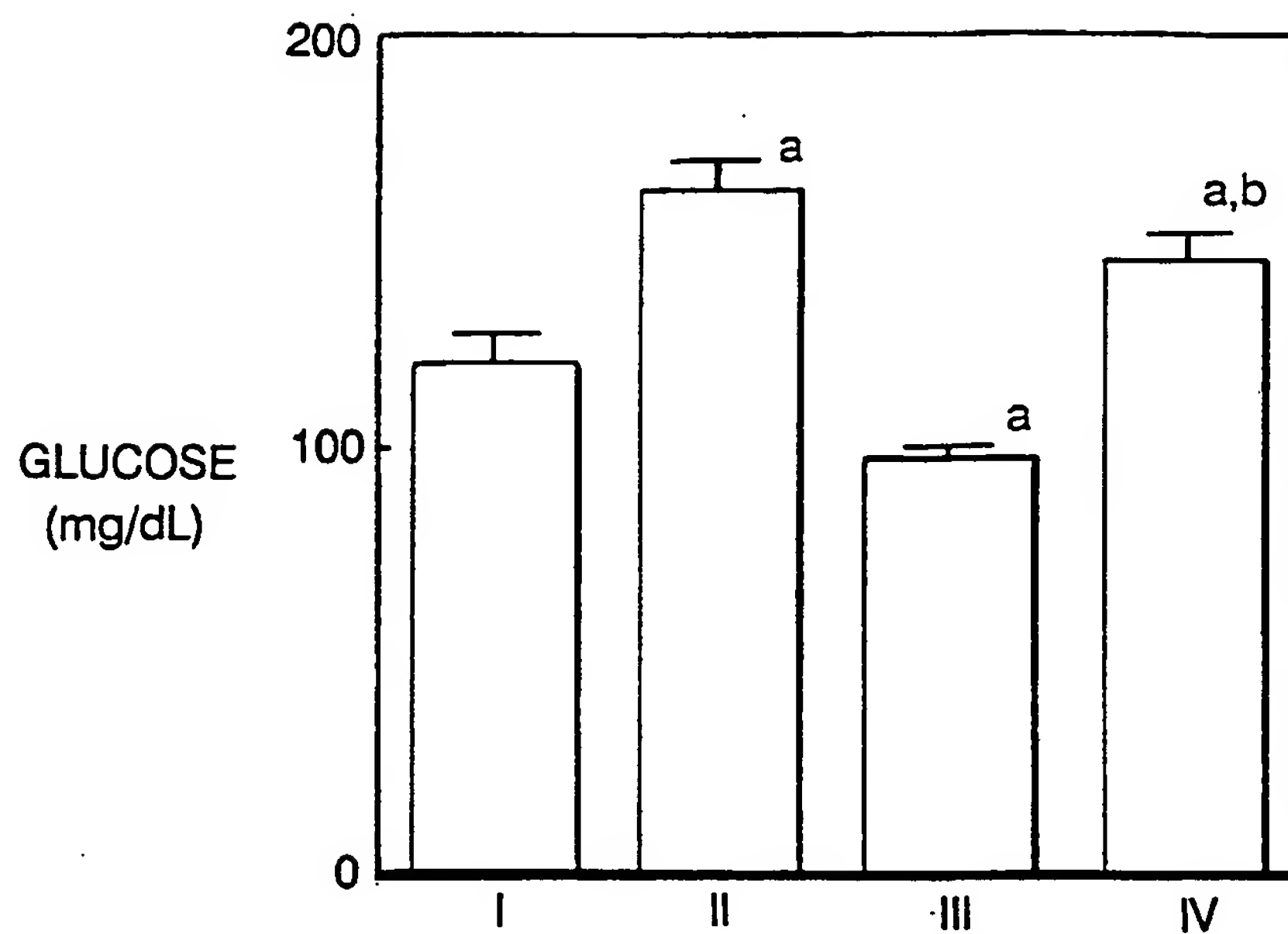


FIG. 7A

a = $p < 0.5$ vs control
 b = not significant vs rat amylin

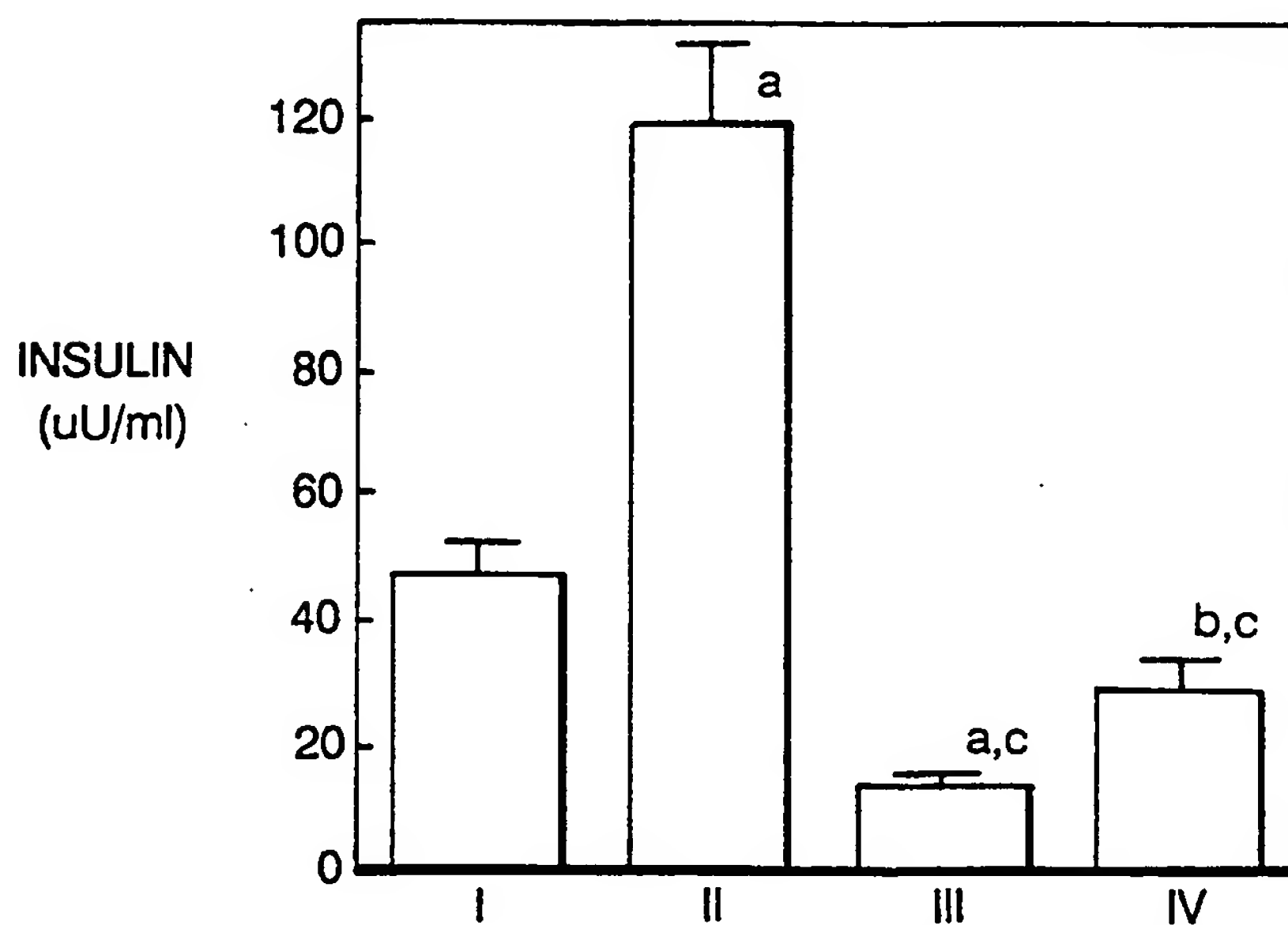


FIG. 7B

a = $p < 0.05$ vs control
 b = not significant vs control
 c = $p < 0.05$ vs rat amylin

I = Saline
 II = Rat amylin (50µg)
 III = [Anb 2,7] rat amylin
 (1-23)-NH² (100µg)
 IV = [Anb 2,7] rat amylin (1-23)-NH²
 (100µg) plus Rat amylin (50µg)

SUBSTITUTE SHEET (RULE 26)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/05282

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 37/02; C07K 7/06, 7/08

US CL :530/326; 514/13

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/326; 514/13

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS, STN

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Proceedings of the National Academy of Science USA, Vol. 85, issued 1988, Cooper et al, "Amylin found in amyloid deposits in Human Type 2 Diabetes Mellitus may be Hormone that Regulates Glycogen Metabolism in Skeletal Muscle", pages 7763-7766, see entire document.	1-13
A	Proceedings of the National Academy of Science USA, Vol. 84, issued June 1987, Westermarck et al, "Amyloid Fibrils in Human Insulinoma and Islets of Langerhans of the Diabetic Cat Are Derived From A Neuropeptid-like Protein Also Present in Normal Islet Cells", pages 3881-3885, see entire document.	1-13

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means		
"P" document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

01 AUGUST 1994

Date of mailing of the international search report

15 AUG 1994

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/05282

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Biochemical and Biophysical Research Communications, Vol. 160, No. 2, issued 28 April 1989, Ohsawa et al, "Islet Amyloid Polypeptide Inhibits Glucose-Stimulated Insulin Secretion From Isolated Rat Pancreatic Islets", pages 961-967. see entire document.	1-13
A,P	US, A, 5,266,561 (COOPER ET AL) 30 November 1993, see entire document.	1-13